Sergi Rodà Llordés
Review
Degree in Biochemistry
2017-18

The cryo-EM revolution

Structural perspective of splicing

Universitat Autònoma de Barcelona

1 Background

Until recently, the study of the splicing was mainly based on biochemical and genetic studies due to the fact that the big size of spliceosome limited the solving of its structure. Right now, this difficulty has been surpassed and the cryo-EM has been the reason.

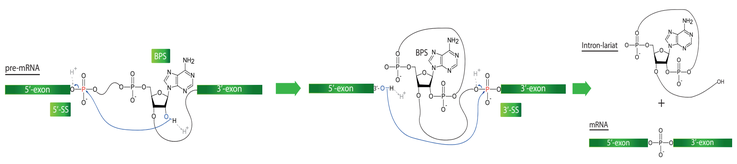
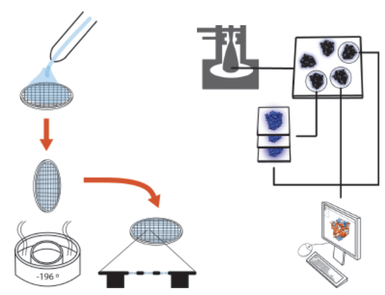


Diagram of the two splicing transesterification reactions

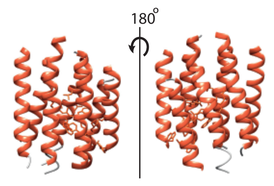
2 Objectives and methodology

- To review and see the progress of cryo-EM as a structural biology technique.
- To analyze the state of the art of splicing in structural terms and its advance thanks to cryo-EM.
- To find related literature, specially the articles concerning the structures of the different spliceosome intermediates.
- Generation of the structures and the images using UCSF Chimera and Adobe Illustrator CC softwares.

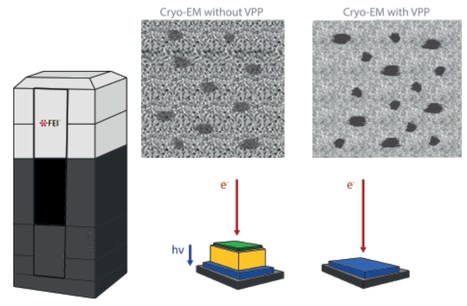
3 Cryo-EM overview



Vitrification method (Dubochet, 1981-88) and single-particle analysis (Frank, 1981)

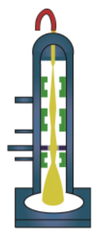


Structure of Bacteriorhodopsin at a 3,5 Å resolution (Henderson, 1990) → PDB: 1BRD

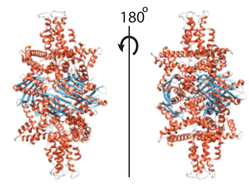


Latest technology: Titan Krios (2005), Direct Electron Detectors (2011) and Volta Phase Plate (2014)

First electron microscope by Ernst Ruska (1933)



Single-particle cryo-EM breaks 2 Å resolution barrier (Merk, 2016) → PDB: 5K12

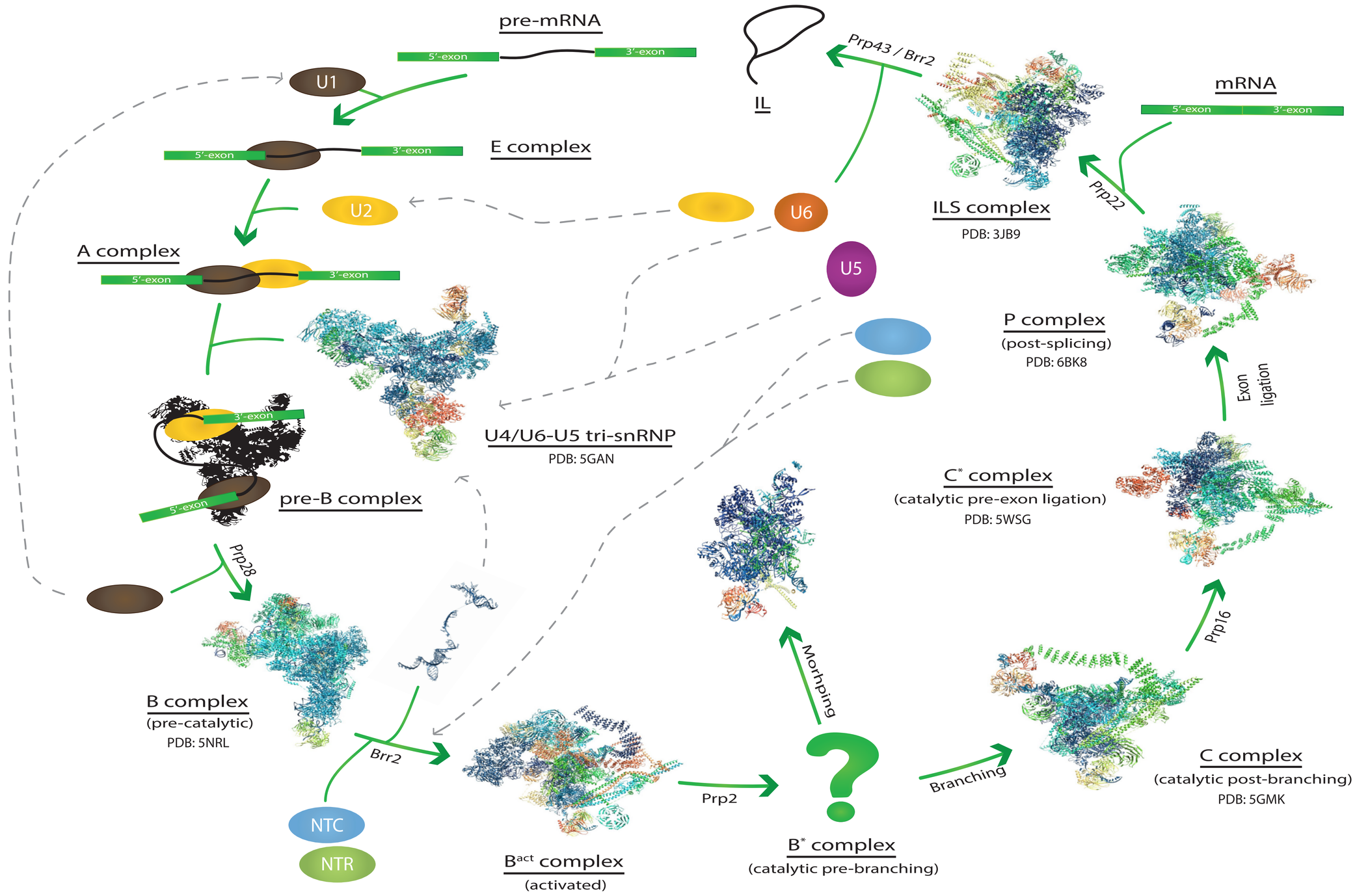


Development of cryo-EM for the high-resolution structure determination of biomolecules in solution (Nobel Prize in Chemistry, 2017)

Taken from [4].

Structural elucidation of splicing cycle by single-particle cryo-EM (2015-2018)

4 Splicing cycle



5 Conclusions

- The cryo-EM revolution is not ceasing anytime soon, being the 2017 Nobel Prize in Chemistry the initial point of it. Furthermore, the improvement in the resolution of the obtained structures and the technique does not seem to stop either.
- The elucidation of the splicing cycle from a structural point of view is a quantum leap in the field of molecular biology. Upcoming experiments essaying to obtain the supraspliceosome structure at high-resolution and to find high-affinity ligands for the different spliceosome intermediates must be the next step.
- The acquisition of the B* complex structure seems a difficult task since its catalytic transition function makes it ephemeral.

6 References

1. Glaeser RM. How good can cryo-EM become? Nat Methods. 2016 Jan 1;13(1):28–32.

2. Neumann E, Farias Estrozi L, Effantin G, Breyton C, Schoehn G. Prix Nobel de Chimie 2017 : Jacques Dubochet, Joachim Frank et Richard Henderson. médecine/sciences. 2017 Dec 20;33(12):1111–7.

3. Cressey D, Callaway E. Cryo-electron microscopy wins chemistry Nobel. Nature. 2017 Oct 4;550(7675):167–167.

4. The Nobel Prize in Chemistry 2017. Available from: https://www.nobelprize.org/nobel_prizes/chemistry/laureates/2017/

5. Fica SM, Nagai K. Cryo-electron microscopy snapshots of the spliceosome: structural insights into a dynamic ribonucleoprotein machine. Nat Struct Mol Biol. 2017 Oct 5;24(10):791–9.

6. Shi Y. Mechanistic insights into precursor messenger RNA splicing by the spliceosome. Nat Rev Mol Cell Biol. 2017 Sep 27;18(11):655–70.

7. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, et al. UCSF Chimera--a visualization system for exploratory research and analysis. J Comput Chem. 2004 Oct;25(13):1605–12.

8. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, et al. The Protein Data Bank. Nucleic Acids Res. 2000 Jan 1;28(1):235–42.

Image at the background of the poster: Cryoplunge™3 and Solarus® 950 | Microscopy and Analysis [cited 2018 May 30]. Available from: https://microscopy-analysis.com/editorials/editorial-listings/cryoplunge™3-and-solarus®-950